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Akhavan-Tafti

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[54] **METHODS OF SYNTHESIZING LABELED
 POLYNUCLEOTIDES BY LIGATION OF
 MULTIPLE OLIGOMERS**

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[*] **Notice:** This patent is subject to a terminal disclaimer.

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 435/91.52; 536/25.3; 536/25.32

[58] **Field of Search** 536/25.3, 25.32;
 435/6, 91.1, 91.2, 91.5, 91.52

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,403,708	4/1995	Brennan	435/6
5,478,724	12/1995	Morse	435/5
5,663,062	9/1997	Sorge	435/91.1
5,695,933	12/1997	Schalling	435/91.52
5,750,341	5/1998	Macevitz	435/6
5,770,367	6/1998	Southern	435/6
5,888,731	3/1999	Yager	435/6

FOREIGN PATENT DOCUMENTS

88311741 6/1989 European Pat. Off. .

OTHER PUBLICATIONS

S. Dubiley, E. Kirilov, Y. Lysov, A. Mirzabekov Nucl. Acids Res., 25, 2259-2265 (1997).

R. Handley, H. Akhavan-Tafti, A. P. Schaap, J. Clin. Ligand Assay, 20(4) 302-312 (1997).

K.D. James, A.D. Ellington, Chemistry & Biology 4, 595-605 (1997).

T. Kaczorowski, W. Szybalski, Gene 179, 189-193 (1996).

L.E. Kotler, D. Zevin-Sonkin, I.A. Sobolev, A.D. Beskin
 L.E. Ulanovsky, Proc. Natl. Acad. Sci. USA, 90, 4241-4245 (1993).

T. Li, K.C. Nicolaou, Nature, 369, 218-221 (1994).

C.E. Pritchard, E.M. Southern, Nucl. Acids Res., 25, 3403-3407 (1997).

D. Sievers, G. Von Kiedrowski, Nature, 369, 221-224 (1994).

W. Stemmer, A. Cramer, K.D. Ha, T.M. Brennan, H.L. Heyneker, Gene, 164, 49-53 (1995).

D.Y. Wu, R.B. Wallace, Genomics, 4, 560-569 (1989).

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[57] **ABSTRACT**

Methods of synthesizing polynucleotides are disclosed involving the simultaneous ligation of a set of oligomer 5'-phosphates onto a template-bound primer. The set of these oligomers can be preselected to contain oligomers which are complementary to the template strand or the oligomers can be supplied as a library and allowed to self select. The synthesis by ligation can proceed unidirectionally or bidirectionally from the primer and can be used to synthesize both strands simultaneously by the use of two primers. The ligation is preferably performed with a ligase enzyme. The methods of synthesis are useful in a variety of applications, including cloning, amplification, labeling, diagnostic assays, mutation analysis and screening, gene expression monitoring and sequence analysis.

23 Claims, 6 Drawing Sheets

